

This is a repository copy of *Host-parasite dynamics in Chagas disease from systemic to hyper-local scales*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/164726/>

Version: Published Version

Article:

Pérez-Mazliah, Damián orcid.org/0000-0002-2156-2585, Ward, Alexander I and Lewis, Michael D (2020) Host-parasite dynamics in Chagas disease from systemic to hyper-local scales. *Parasite immunology*. e12786. ISSN 1365-3024

<https://doi.org/10.1111/pim.12786>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Host-parasite dynamics in Chagas disease from systemic to hyper-local scales

Damián Pérez-Mazliah¹  | Alexander I. Ward² | Michael D. Lewis² 

¹York Biomedical Research Institute, Hull York Medical School, University of York, York, UK

²Department of Infection Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

Correspondence

Michael D. Lewis, Department of Infection Biology, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK.

Email: michael.lewis@lshtm.ac.uk

Funding information

Medical Research Council, Grant/Award Number: MR/N013638/1 and MR/R021430/1

Abstract

Trypanosoma cruzi is a remarkably versatile parasite. It can parasitize almost any nucleated cell type and naturally infects hundreds of mammal species across much of the Americas. In humans, it is the cause of Chagas disease, a set of mainly chronic conditions predominantly affecting the heart and gastrointestinal tract, which can progress to become life threatening. Yet around two thirds of infected people are long-term asymptomatic carriers. Clinical outcomes depend on many factors, but the central determinant is the nature of the host-parasite interactions that play out over the years of chronic infection in diverse tissue environments. In this review, we aim to integrate recent developments in the understanding of the spatial and temporal dynamics of *T. cruzi* infections with established and emerging concepts in host immune responses in the corresponding phases and tissues.

KEYWORDS

cell mediated immunity, Chagas disease, humoral immunity, in vivo imaging, innate immunity, trypanosomes

1 | *TRYPANOSOMA CRUZI*: A FORMIDABLE Foe

Trypanosoma cruzi, the causative agent of Chagas disease (American trypanosomiasis), is an extraordinarily versatile parasite. Its wild transmission cycles across the Americas are maintained by over 100 species of haematophagous triatomine bugs. Chagas disease is a zoonosis, and *T. cruzi* infects diverse mammal reservoir species, including marsupials, bats, rodents, ungulates, carnivores (including domestic cats and dogs), armadillos, pilosans and primates.¹ *T. cruzi* undergoes regulated morphological transitions involving at least four developmental forms, each with a distinctive biology, for example cell structural features, modes of motility, surface protein coats² and metabolic programmes.³ The epimastigote form replicates in the

vector's gut, then differentiates to a highly motile form, the metacyclic trypomastigote, which invades mammalian host cells after transmission. Potentially any nucleated cell type may be parasitized in any tissue the trypomastigote can reach.⁴⁻¹⁶ After invasion and escape from a parasitophorous vacuole into the cytosol, another transition occurs to the amastigote form, which replicates repeatedly and then differentiates to generate a population of pleomorphic tissue/bloodstream form trypomastigotes. These are released into the extracellular space, from where they may infect a new cell, in some cases after migration to the bloodstream. Alternatively, in the event they are taken up in a triatomine blood meal, they can complete the cycle by differentiating into epimastigotes.

Mounting evidence shows *T. cruzi*'s life cycle is considerably more complex than the textbook view. Findings include epimastigote-like

Damián Pérez-Mazliah and Alexander I. Ward contributed equally.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Parasite Immunology* published by John Wiley & Sons Ltd

forms in mammals, asynchronous replication and trypomastigogenesis, asymmetric divisions, reversible transitions and formation of apparently quiescent or dormant amastigotes.¹⁷⁻²² The morphological, antigenic and spatial variability, combined with active evasion strategies, presents a formidable challenge to the mammalian immune system. Nevertheless, most infections resolve to a stable chronic equilibrium of parasite replication and suppression via a combination of sustained antibody and type 1 cellular responses. The majority of people (>95%) survive acute infection and progress to a chronic, asymptomatic phase. Chagas cardiomyopathy is then estimated to develop at a rate of ~2% per year.²³ Disorders of the gastrointestinal (GI) tract develop in a smaller proportion of cases, sometimes in combination with cardiac disease.²⁴ Why Chagas pathology only affects a limited subset of tissues, in only a specific subset of infected people, is one of the longest-standing and most important unanswered questions in the field.

In this review, our aim is to integrate recent developments in the understanding of the spatial and temporal dynamics of *T. cruzi* infections with established and emerging concepts in host immune responses in the corresponding phases and tissues. The result is a view that parasite persistence occurs in a small number of privileged tissues alongside highly competent, *T. cruzi*-specific systemic responses, suggesting a substantial degree of compartmentalization, even within tissues. The low-level, yet perpetual chronic inflammation has the potential to become pathological, dependent on largely undefined host, parasite and environmental factors. Thus, progress in the development of anti-parasitic drugs, adjunct treatments, immunotherapies and vaccines is likely to require a much better understanding of the molecular and cellular determinants of *T. cruzi* persistence at the tissue-specific and even hyper-local, intra-tissue scale.

2 | ADVANCES IN STUDIES OF TISSUE-SPECIFIC INFECTION DYNAMICS

While *T. cruzi* must spend some time in extracellular environments of the blood and interstitial fluid to sustain infections and ensure transmission, it is predominantly an intracellular parasite of solid organs. Consequently, most of what is known of the cells and tissues targeted by *T. cruzi* comes from experimental animal studies. It is difficult to obtain robust data on tissue distribution in human patients, although post-mortem, transplant and biopsy results tend to be consistent with animal models. The mouse is the species of choice, but other rodents, rabbits, dogs and nonhuman primates have also demonstrated utility.²⁵ Tissue-specific parasite loads can be measured by a range of direct and indirect methods (reviewed in 26). Developments in real-time bioluminescence imaging methods have underpinned much recent progress in understanding *T. cruzi* infection dynamics.^{4,5,27-31} These systems are based on transgenic parasites expressing luciferases, enabling analysis of light signals emitted by parasites in discrete anatomical locations. Major advantages include greatly reduced tissue sampling bias and the ability to monitor

individual mice over time. Bioluminescence lacks the resolution necessary to visualize parasites at individual cell scale, but this can be achieved using parasites expressing fluorescent reporters,^{21,32,33} an approach that becomes particularly powerful when luciferase-fluorescence fusion proteins are employed (Figure 1).^{22,34} The possibility to integrate these imaging methods with analyses of concomitant immune responses³⁵ holds considerable promise for advancing our understanding of *T. cruzi*-host interactions.

3 | STAGE 1: *T. CRUZI* SYSTEMIC COLONIZATION AND INNATE RESPONSES

In vectorial transmission scenarios, infection results from contamination of the triatomine bite wound or of mucosal membranes with trypomastigotes present in the bug's faeces. Transmission may also occur orally, via contaminated food or drink, *in utero*, and by blood transfusion and organ transplant. Several mechanisms of host cell invasion have been described and reviewed elsewhere.³⁶ In humans, oedema with intense mononuclear infiltrate at the entry site in the skin (chagoma) or eye (Romaña's sign) indicates an initially very localized infection.³⁷ However, the true extent of trypomastigote dissemination is not clear and surprisingly little is known at the cellular level about the actual sites of primary invasion and the first cycle of intracellular parasite replication, which lasts approximately 1 week. Experimental animal studies indicate that the route of inoculation is a key factor. Intra-peritoneal injection results in similar parasite numbers in diverse tissues after 6 days.³⁸ Conversely, oral transmission results in highly localized infections in the stomach or nasomaxillary tissues^{31,38,39} with initial infection of the local mucosal epithelium.⁴⁰ Similarly, after conjunctival inoculation, parasites first invaded and replicated in the mucosal epithelium of the nasolacrimal ducts and nasal cavities.⁴¹

At the end of the first intracellular cycle, trypomastigotes are released and the infection disseminates widely. *T. cruzi* is pan-tropic in the acute phase of infection (reviewed in 26). However, the relative intensity of infection in different cell or tissue types again varies depending on the inoculation route and inoculum size, as well as intrinsic factors such as replication rate and capacity for dissemination. Sites reported to harbour the highest acute infection intensities include skeletal, smooth and cardiac muscle and adipose tissues. Some studies have described *T. cruzi* strains with an increased capacity to parasitize mononuclear phagocytes^{13,42,43} or to cross the blood-brain barrier.^{12,44,45}

3.1 | Sensors

The host response to *T. cruzi* primary infection is considered to be markedly delayed by comparison with model intra-cytosolic pathogens.^{46,47} The main features of the immediate response are the induction of type I interferon signalling, and recruitment of neutrophils, macrophages and Natural Killer (NK) cells.⁴⁸ Ca²⁺ mobilization,

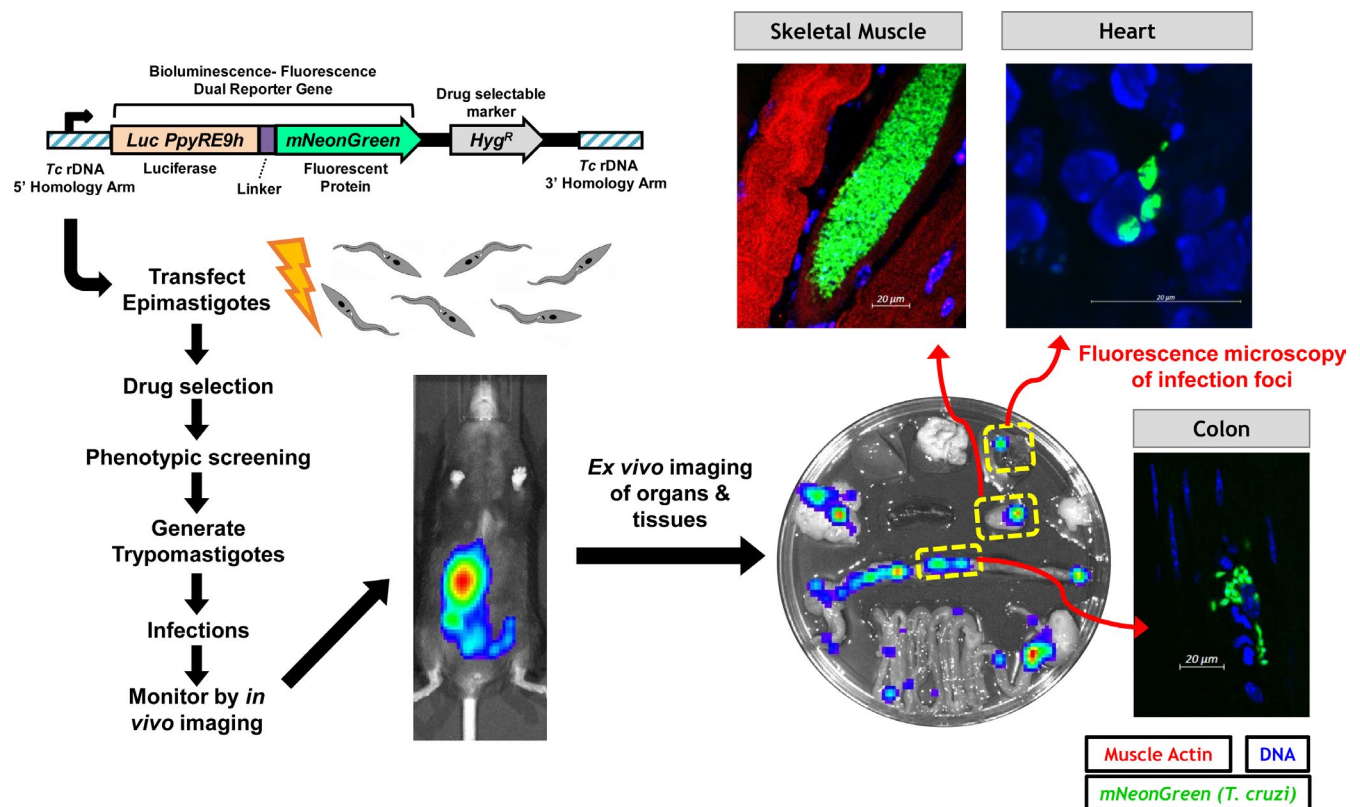


FIGURE 1 Visualization of *Trypanosoma cruzi* infection foci in vivo using bioluminescent-fluorescent dual reporter parasites

associated with invasion of myeloid cells, can activate the transcription factor NFATc1, leading to interferon gamma (IFN γ) production by NK cells and dendritic cell (DC) maturation.⁴⁹ *T. cruzi* also produces multiple B-cell mitogens that directly trigger a robust T-independent B-cell activation.^{50–52}

Few canonical pathogen-associated molecular patterns (PAMPs) are conserved in *T. cruzi*. The best characterized innate pattern recognition receptors (PRRs) for *T. cruzi* PAMPs are Toll-like receptor (TLR) 2 and 9. These recognize, respectively, the glycosylphosphatidylinositol (GPI) anchor of parasite surface proteins and parasite DNA, specifically unmethylated CpG motifs.^{53,54} TLR2 + 9 double-knockout mutant mice suffer higher parasitaemias and significantly increased mortality rates (50% by day 50) compared to wild-type controls.⁵⁴ Mice lacking both MyD88 and TRIF, thus rendered incapable of any TLR-mediated responses, have uncontrolled parasitaemia and 100% mortality by 18 days of infection.⁵⁵ This may be explained by the additional involvement of TLR4 and TLR7, recognizing parasite glycosylphospholipids and RNA, respectively.^{56–58}

Many *T. cruzi* surface proteins are extensively glycosylated,² and several host galectins (a widely expressed family of carbohydrate-binding proteins) are able to bind them.⁵⁹ Interactions involving several different galectins may actually help *T. cruzi* bind to and enter host tissues,^{60,61} but this does not appear to directly trigger any anti-parasitic effector activity. As an occupant of the host cell cytoplasm, it is likely that *T. cruzi* triggers cytosolic sensors. The best known candidate systems centre on NOD-like receptors (NLR). Mice lacking the NOD1 receptor suffer 100% mortality to acute

T. cruzi infection, although the mechanism explaining this remains obscure.⁶² Studies also support a parasite-suppressive role for the related receptor, NLRP3 and downstream components of the inflammasome complex that drives IL-1 β and IL-18 secretion,^{63–65} although, as with NOD1, it is not clear if this involves direct sensing of *T. cruzi* in vivo.

The majority of studies of innate immunity to *T. cruzi* have focussed on responses in myeloid cells, especially macrophages, yet these could represent only a minor subset of the parasite's early targets. Transcriptomic analysis of in vitro-infected human fibroblasts revealed that inflammatory cytokine expression peaked 24 hours post-infection and the TLR-independent type I IFN response became the dominant signature by 72 hours, which was suggested to promote, rather than inhibit, the infection.⁶⁶ Trypomastigotes have a diverse secretome comprising proteins in native form and also as cargoes in shed extracellular vesicles.^{67–70} Important research questions to address include whether and at what point these are relevant for triggering PRRs in vivo and whether there are equivalent processes for intracellular amastigotes.

After the first cycle of replication ends, host cells rupture and trypomastigotes escape into the extracellular environment. At this point, trypomastigotes may invade local tissue cells, enter infiltrating leucocytes or migrate via the blood or lymphatics to other tissues. The factors governing the parasite's propensity to stay local or not remain obscure. Host cell rupture releases intracellular material rich in danger-associated molecular patterns (DAMPs), further stimulating innate signalling via TLRs as well as, for example, degranulation

of nearby mast cells and activation of myeloid cells. Recently, sensing of oxidized DNA in extracellular vesicles via cyclic GMP-AMP Synthase (cGAS) was identified as an important DAMP recognition mechanism for macrophage activation.⁷¹ As a eukaryote, *T. cruzi* has endogenous orthologues of many mammalian DAMPs, potentially blurring the boundaries between DAMPs and PAMPs. For example, recombinant *T. cruzi* High Mobility Group B (TcHMGB) protein can induce production of nitric oxide (NO) in macrophages in vitro and expression of genes encoding inflammatory cytokine genes in vivo.⁷²

3.2 | Signal mediators and amplifiers

A plethora of cross-talking signalling pathways are activated downstream of the PAMP/DAMP sensors described above. Signalling converges on a set of transcription factors (NF- κ B, AP-1, IRF3), which results in production of inflammatory cytokines.⁷³⁻⁷⁶ Critical amongst these are the IL-12 family, IFN γ and TNF- α , the canonical drivers of type 1 immune responses required to tackle intracellular infections. IL-12 is essential for the early activation of recruited natural killer cells and their production of IFN- γ ; both these cytokines are indispensable for control of parasite loads and avoidance of acute mortality.⁷⁷ TNF- α is also essential for survival of the acute stage.⁷⁸ IFN γ and TNF- α activate parasite destructive effector mechanisms via autocrine and paracrine signalling. Beyond the canonical IL-12-IFN γ axis, signalling through the IL-1 receptor is essential for early (10 days p.i.) induction of the myocarditis needed to control heart parasitism.⁶⁵

The local tissue response is amplified via chemokine-driven recruitment of inflammatory monocytes, macrophages, neutrophils and, eventually, antigen (Ag)-specific CD4⁺ helper T and CD8⁺ cytotoxic T lymphocytes (Th and CTL) to the site of infection.⁷⁹ Microvascular plasma leakage into parasitized tissues is promoted further by activation of mast cells and the kallikrein-kinin system (KKS), via a mechanism involving cruzipain, a parasite-derived cysteine protease.⁸⁰ The resulting tissue oedema and upregulation of associated receptors on cardiomyocytes may increase specific susceptibility to heart invasion as the infection progresses.⁸¹

3.3 | Innate effectors and their evasion

The infection, cell necrosis and associated inflammatory signalling result in the activation of a range of innate effector mechanisms. There is some evidence from analysis of Beclin-1-deficient mice that host cell autophagy can provide some marginal early restraint on parasite replication.⁸² Epimastigotes are complement-sensitive but trypomastigotes have effective molecular mechanisms providing resistance to complement-mediated lysis.⁸³ Infiltrating NK cells, in addition to being major producers of IFN γ , may have direct parasitocidal effects involving the release of cytotoxic granules.⁸⁴

An unusual population of innate-like CD8⁺ T cells with activation characteristics of (e.g. production of granzyme A and IFN γ) expands

in the thymus of *T. cruzi*-infected mice. These cells appear to be driven by antigen-independent mechanisms, and adoptive transfer experiments of thymocytes from infected mice suggest they might provide protection from otherwise lethal challenge⁸⁵; however, the underlying mechanisms conferring this protection remain to be elucidated.

Reactive oxygen and nitrogen species (ROS, RNS) are principal effectors for *T. cruzi* control. These are generated by IFN γ /TNF- α -activated macrophages and via diverse other mechanisms in non-phagocytes and extracellular compartments.⁸⁶ They are a significant cause of collateral damage in infected tissues, but high levels are necessary because *T. cruzi* has an extensive and highly effective antioxidant defence system.^{86,87} ROS can even promote *T. cruzi* replication, by a mechanism proposed to depend on the increased availability of intracellular Fe²⁺ ions that the parasite can utilize.⁸⁸ Nitric oxide (NO) is directly parasitocidal in vitro,⁸⁹ and inducible NO synthase (iNOS) is essential for in vivo parasite control in some models,^{62,77,90} although not in others.^{63,91}

Despite the plethora of innate responses, the overall effectiveness of *T. cruzi*'s evasion mechanisms renders it debatable whether they actually have any meaningful impact on most infections, apart from the induction and conditioning of the adaptive response (see below). Indeed, *T. cruzi* infections are 100% lethal in mice that are genetically incapable of mounting adaptive responses (SCID, RAG, nude)⁹²⁻⁹⁴ and bioluminescence imaging studies show that parasite growth in such mice is close to exponential.⁴

4 | STAGE 2: ADAPTIVE RESPONSES TAKE CONTROL

The infection usually peaks, in terms of total parasite numbers and the extent of tissue dissemination, at a point between 2 and 3 weeks post-infection. Over the following weeks, parasite loads are reduced by several orders of magnitude by a highly effective adaptive immune response. Although they are ultimately thought to be non-sterilizing in virtually all cases,⁹⁵ it is worth reviewing the key features at the systemic level before we consider the tissue-specific host-parasite dynamics at play in the chronic phase. We also refer readers to more in-depth reviews of adaptive immunity in Chagas disease.^{46,96,97}

4.1 | T- and B-cell activation

T. cruzi cycles between the cytosolic and extracellular compartments and, accordingly, its control is critically dependent on the generation and deployment of Ag-specific CTL to infected tissues and antibody production by B cells. This is evidenced by relevant gene disruption and antibody-mediated depletion experiments in mice.⁹⁸⁻¹⁰² Mature DCs in the spleen and lymph nodes draining infected tissues, conditioned by the inflammatory environment, activate parasite Ag-specific CD8⁺ and CD4⁺ T cells from the naïve pools.^{47,103}

Activated T cells then migrate to sites of infection to exert effector mechanisms or, in some cases, begin differentiation to memory subsets.^{102,104} A number of factors may impinge on the quality and magnitude of the T-cell response, including parasite-driven immature thymocyte apoptosis¹⁰⁵ and direct and indirect modulation of DC-T cell interactions.¹⁰⁶⁻¹⁰⁹ In terms of antigen specificity, the murine T-cell repertoire is focussed mainly on a small number of immunodominant epitopes from highly expressed surface proteins,¹¹⁰⁻¹¹² but in humans there is evidence of a broader hierarchy^{97,113} and immunodominance appears not to directly contribute to chronicity.

The role of CD4⁺ T cells is not well characterized, but the association between HIV infection and life-threatening acute *T. cruzi* relapse in humans¹¹⁴ indicates they are critical for parasite control. Accordingly, mice that are specifically incapable of mounting CD4⁺ T-cell responses experience 100% acute lethality of *T. cruzi* infection.¹¹⁵ This has been linked to loss of support for parasite-specific CD8⁺ T-cell cytotoxicity against intravenous delivered splenocytes loaded with parasite antigens from the ASP-2 gene,⁹⁹ but not in similar experiments using trans-sialidase peptides.¹¹⁶ This may reflect differing requirements for T-cell help depending on immunodominance hierarchies.⁹⁷ Nevertheless, the majority of CD4⁺ T cells develop a protective Th1 profile and contribute further to the abundance of type 1 cytokines, particularly IFN γ .^{93,117-119} Broader phenotypic diversity does develop alongside Th1 predominance, including minor Th17, Th1/Th17 intermediate and possibly Th2 subsets in some circumstances.^{118,120-124} There is no clear consensus on the relevance of these to parasite control and immunopathogenesis, but this is an active area of research. The CD4⁺ T cells that provide B-cell help are termed follicular helper T cells (Tfh), and represent a distinct CD4⁺ T-cell programme regulated by the master transcription factor Bcl6. Although activation of Tfh responses to *T. cruzi* infection has not been explored in detail, it is reasonable to hypothesize that they are required for the production of parasite-specific antibodies and ultimate control of the infection. In line with this, IL-6, which supports Tfh differentiation,^{125,126} is required for the control of parasitaemia and splenocyte recall response to parasite antigens,¹²⁷ but not for T-cell independent polyclonal activation of B-cell responses.⁵¹

The initial B-cell response in the spleen is estimated to be at least 10-fold higher as compared to LNs draining infected tissues,⁵² and a robust *T. cruzi*-specific antibody response is still generated there alongside the aforementioned polyclonal B-cell activation and non-specific hyper-gammaglobulinaemia. The parasite-specific antibody response is presumably driven by B-cell activation involving T-cell collaboration because it is accompanied by a robust germinal centre B-cell response and production of parasite-specific class-switched antibodies.⁵² The specific and non-specific splenic B-cell responses appear to be either differentially regulated or carried out by different B-cell compartments because only the latter depend on the cytokine B-cell activating factor (BAFF).¹²⁸

Activation of auto-reactive T- and B-cell clones, the latter leading to the production of autoantibodies, is a well-described phenomenon during *T. cruzi* infection.¹²⁹ Polyclonal B-cell activation, host molecular mimicry by parasite proteins and bystander activation

caused by tissue damage have been postulated as underlying mechanisms.¹²⁹ There is broad evidence and consensus that parasite persistence is required to sustain these autoimmune responses.¹³⁰⁻¹³² Nevertheless, the significance of autoantibodies and auto-reactive T cells for Chagas disease pathogenesis and the mechanisms involved in their production during *T. cruzi* infection remain major unresolved questions.

It has been suggested that the non-specific polyclonal B-cell activation contributes to delay the generation of *T. cruzi*-specific B-cell responses, thus contributing to parasite escape and establishment of chronic infections.^{133,134} Polyclonal B-cell activation is associated with rapid, innate-like production of IL-17 and IL-10^{135,136} but the wider relevance is unclear as both protective¹³⁶⁻¹³⁸ and deleterious^{51,52,135} roles for such innate-like B-cell responses have been documented in different models. The infection also causes a transient, yet marked loss of immature B cells in the bone marrow in experimental mouse models, possibly further compromising the response.¹³⁹

The kinetics of the adaptive response depend to some extent on the early parasite load,⁹⁹ but in most cases, it coincides with the second or third intracellular cycle of parasite replication and is considered relatively delayed.⁴⁷ Nevertheless, substantial immune memory can be generated quite rapidly: mice whose infections were cured by benznidazole anti-parasitic chemotherapy starting 4 or 14 days after infection were then able to restrict acute parasite loads in challenge infections by 85% and >99%, respectively.³⁵ Notably though, very few of these animals achieved sterile cure and they progressed to chronic phase infections that were comparable to primary infections in naïve mice. This raises important questions about what is mediating memory responses to secondary infections, for example whether they are T cell-dependent or independent.

4.2 | Adaptive effector mechanisms

Lymphocytes contribute to control of *T. cruzi* by production of type 1 cytokines that amplify the prior, innate ROS and RNS production in infected tissues. Their signature, direct effector mechanisms are also crucial. These include the principal CTL effector pathways, namely perforin-mediated delivery of granzymes and FasL-induced apoptosis. In particular, granzymes cause fatal oxidative damage to *T. cruzi*, which can be mitigated by ROS scavenging drugs or overexpression of parasite antioxidant genes.¹⁴⁰ This may potentially be accelerated in humans by granulysin-mediated delivery of granzymes directly into intracellular parasites themselves.¹⁴⁰ Mice do not have a granulysin gene but in most cases still achieve good control of parasite levels, so immune pressure may be more focussed on extracellular amastigotes after host cell apoptosis and on clearance of trypomastigotes. These canonical pathways are essential in some experimental models¹⁴⁰⁻¹⁴² but dispensable in others.^{98,143} The difference is likely explained by other pathways providing sufficient compensatory effector capacity in lower parasite load or virulence scenarios.

T. cruzi-specific lytic and neutralizing antibodies are normally detected in humans and animal models.¹⁴⁴⁻¹⁴⁷ These are mostly produced in the spleen; antibody secretion by bone marrow cells obtained from acutely infected mice is below detection level.⁵² This suggests that either plasma cells generated in secondary lymphoid organs during acute *T. cruzi* infection are unable to migrate to the bone marrow, or that the bone marrow may not sustain plasma cell survival during the early phase of the infection, or that plasma cell homing in the bone marrow is somehow delayed during *T. cruzi* infection. Whether this is a temporary mechanism or extends throughout chronic infection and whether it is a direct mechanism driven by presence of the parasite in the bone marrow are unknown.

Antibodies target extracellular trypomastigotes, but they may also have a role in binding amastigotes released from ruptured host cells, for example, downstream of CTL-mediated lysis. Opsonized parasites are efficiently taken up by tissue-resident macrophages, especially in highly vascularized organs, for example liver, lung, spleen.^{148,149} It is not clear how a subset of trypomastigotes evade this fate to sustain chronic infections. Beyond their role as antibody producers, B cells are also critically required for functional T-cell responses to control *T. cruzi* infection^{102,150-152} and production of cytokines, including IL-17 and IL-10.^{135,136}

4.3 | Deactivating/Regulatory mechanisms

The strong and sustained systemic inflammation, host cell lysis and tissue parasite killing in this control phase cause potentially

dangerous levels of collateral tissue damage. Infections may become overtly symptomatic and in some cases fatal, particularly if the CNS is involved.¹⁵³ Tissue-protective immune regulatory pathways are therefore initiated to dampen the inflammatory response, to the benefit of the remaining parasites, which form the founding populations of the chronic infection reservoirs (Figure 3).

The factor with the strongest evidence for an important regulatory role is probably the cytokine IL-10. Early studies of IL-10 deficiency using high virulence Tulahuen strain parasites reported better control of acute *T. cruzi* parasitaemia at the expense of rapidly fatal (~2 weeks p.i.) pathogenic inflammation, for example TNF- α -mediated toxic shock.^{154,155} More recent studies point to greater complexity. Rôffe *et al* (2012) reported IL-10 was essential to protect against later mortality (3-6 weeks p.i.) associated with poor control of Colombiana strain tissue parasite loads and increased myocarditis intensity. In still lower virulence scenarios, the absence of IL-10 has been associated with reduced CTL effector function but without any increased mortality.¹⁵⁶ Both CD8⁺ and CD4⁺ T cells are IL-10 sources, and a high proportion simultaneously produce IFN γ ,¹⁵⁷ likely supported by IL-27 production¹⁵⁸ and potentially in direct response to parasite shed *trans*-sialidase.¹²³ B cells also produce IL-10,¹³⁵ and overall IL-10 production is lower in B1 B-cell-deficient mice early during infection.¹⁵⁹ CD11b⁺ B1 B cells from asymptomatic, infected individuals show increased capacity to produce IL-10 compared to those with cardiac disease symptoms.¹³⁸ In addition, recent data show that when compared to non-infected donors, chronically *T. cruzi*-infected individuals with cardiac manifestations have an

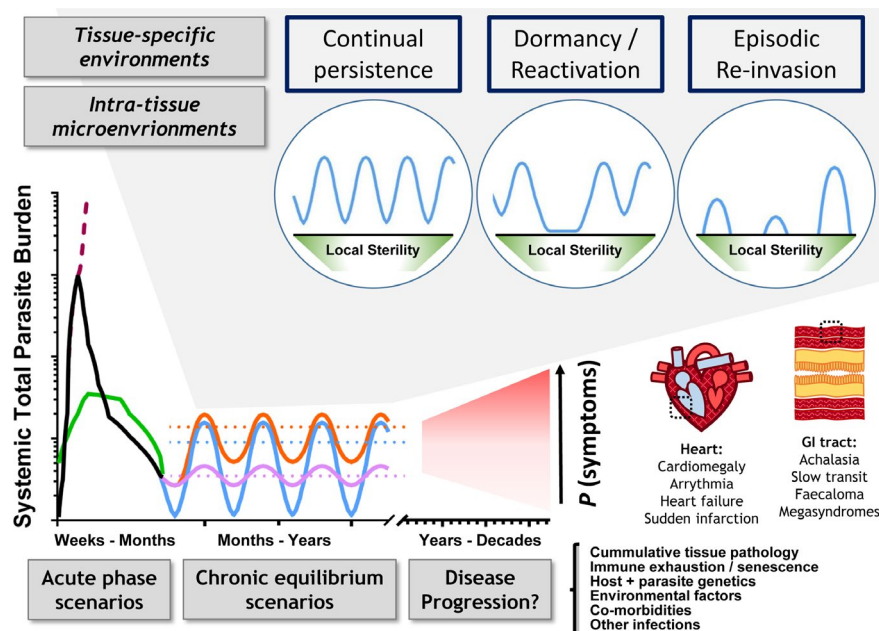


FIGURE 2 Overview of host-parasite interaction dynamics in *Trypanosoma cruzi* infections. Chart illustrates some alternative course of infection scenarios for acute and chronic phase total parasite burdens, feeding into potential clinical outcomes, which range from long-term non-progression to severe Chagas disease affecting the heart and/or gastrointestinal tract. The chronic equilibrium scenarios are the product of many temporally overlapping host-parasite interactions within and between the various organs targeted for infection by *T. cruzi*. Three possible, non-mutually exclusive modes of persistence at the tissue or tissue sub-domain level are illustrated above the chart, continual persistence, dormancy/reactivation and episodic re-invasion.

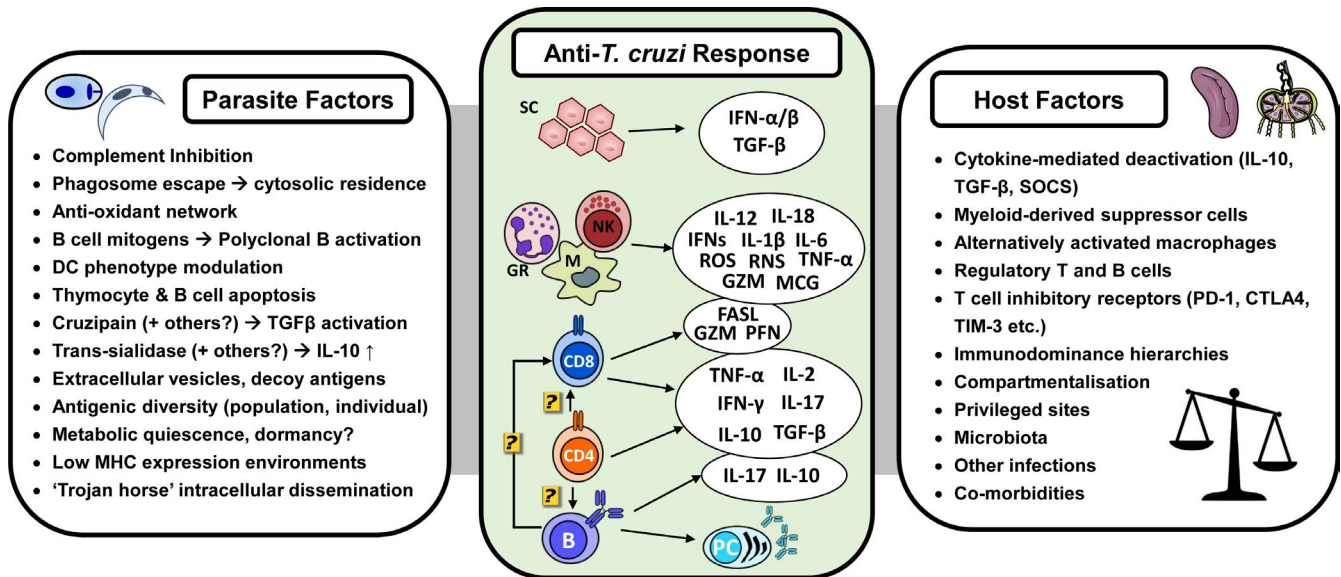


FIGURE 3 Overview of the principal cellular and molecular components of the immune response to *Trypanosoma cruzi* infection. Central panel shows key cell types, cytokines and effector molecules. B, B cell; CD4, helper T cell; CD8, cytotoxic T cell; GR, granulocytes; M, myeloid cells; NK, natural Killer cells; PC, plasma cell; SC, somatic cells. Left and right panels show parasite and host factors potentially contributing to infection chronicity.

increased proportion of immature transitional CD24^{high}CD38^{high} and naïve B cells able to produce IL-10 upon in vitro re-stimulation.¹⁶⁰ This suggests B cell-intrinsic IL-10 signalling might be important to regulate the intense adaptive immune response, as is the case for other parasitic infections,¹⁶¹ but direct mechanistic evidence is required to support this hypothesis.

Transforming growth factor beta (TGF-β), another potent regulatory and tissue-protective cytokine, can be activated from its latent form by a *T. cruzi* protease (cruzipain) in vitro.¹⁶² TGF-β signalling to T cells reduces the risk of late acute mortality, and this appears to involve inhibition of cell proliferation rather than suppression of inflammatory cytokine production.^{106,163} Other factors potentially contributing to early inhibition of adaptive immune effector responses include suppressor of cytokine signalling (SOCS),¹⁶⁴ regulatory CD4⁺ T cells (Tregs)^{106,165} and induction of various regulatory/suppressive myeloid cell phenotypes, such as expression of iNOS-limiting arginase.^{109,166,167}

The overall result of these deactivating pathways is the avoidance of potentially life-threatening levels of inflammation and tissue damage at the expense of incomplete clearance of the infection (Figures 2 and 3). The situation at the tissue-specific level, however, is likely to be more complex because only a subset of tissues serve as privileged sites for *T. cruzi* persistence in the chronic phase.²⁶

5 | STAGE 3: THE 1% AND THE CHRONIC HOST-PARASITE EQUILIBRIUM

In the chronic phase, blood parasitaemia is typically sub-patent and tissue parasite loads are between 0.1% and 1% of their levels in the acute phase.^{4,29} Animal imaging studies⁴ and serial analysis of patient

blood by PCR¹⁶⁸ show infection levels fluctuate over time, pointing to a dynamic equilibrium between intracellular parasite replication, antibody and effector T-cell activity. The state of this equilibrium at the organismal level is the product of many discrete host-parasite interactions within and between multiple tissues (Figure 2). Over time, these interactions can become overtly pathological and subsets of infected people develop a spectrum of symptomatic forms of Chagas disease, as reviewed elsewhere.¹⁶⁹

Given the difficulty in sampling tissue parasites from humans, most of our knowledge on their tissue distribution comes from animal models. These indicate that chronic infection dynamics are shaped by the combination of *T. cruzi* strain and the host's genetic background. It appears that the GI tract, mainly the large intestine and stomach, is a universal site of continual parasite persistence in mice.^{29,30,170} The well-studied parasite strain CL Brener is also commonly detected in the skin of BALB/c mice³⁸ but only sporadically in other sites, for example skeletal muscle, lung, adipose. More virulent parasite strains (eg Brazil, Colombiana, Tulahuen, VFRA) and certain mice (eg C3H) are associated with more disseminated infections, including heart and skeletal muscle localization.^{29,30,170-175} There have been few robust data on the relevant cell types within any of these chronically infected tissues, but recent in vivo imaging analysis at single cell resolution revealed smooth muscle cells as the most frequent targets in the colon.¹⁷⁵

A population of central memory T cells (T_{CM}) is detectable during the chronic phase of infection, which may stem from a lack of parasite antigen in lymph nodes draining non-parasitized organs.^{104,176} These T_{CM} are maintained after benznidazole-mediated cure of chronic infection and provide protection against re-challenge after transfer into naïve mice.¹⁷⁷ Homologous challenge infections, in drug-cured mice themselves, result in acute

parasite loads <1% of those in primary infection controls, and fully sterile protection is seen in around half of the animals.³⁵ The determinants of both categories of protection remain to be defined, but it is likely critically dependent on the T_{CM} population. It is likewise an open question whether T_{CM} -derived effectors contribute to suppression of tissue parasite numbers during chronic infections, particularly in organs subject to cycles of episodic re-invasion and clearance.

The mechanisms of immune evasion sustaining the host-parasite equilibrium during perpetual chronic infection are not necessarily the same as those that prevent sterile clearance in the acute to chronic transition, which resemble a conserved host tissue-protective, anti-inflammatory programme. They are also harder to study, both from the parasite perspective, owing to the scarcity of *T. cruzi* foci in tissues, and from the host perspective because of the need for conditional intervention techniques that can be applied after acute infections have been brought under control. The essentiality of CD8⁺ T cells for continued suppression of parasite numbers in the chronic phase is reasonably clear. Parasite loads rebound rapidly upon treatment with anti-CD8 antibodies, almost to the level seen with pan-adaptive immunosuppression using cyclophosphamide,^{174,178} although depletion of CD8⁺ NK cells and DCs may contribute to the relapse, in addition to CTLs. Unlike in the acute phase, experimental anti-CD4 treatment has no effect on chronic parasite loads.¹⁷⁸ Nevertheless, severe reactivation of Chagas disease in HIV co-infected patients indicates CD4⁺ T cells are vital for control and the frequent presentation of meningoencephalitis points to a specific role in protection against invasion of the CNS.¹¹⁴

There are various non-exclusive hypotheses for how a small sub-population of parasites reliably evades sterilization in the face of the sustained adaptive immune pressure. However, in our view none currently has compelling evidence supporting a mechanistic explanation so this will remain an active area of investigation.

5.1 | Antigenic diversity

African trypanosomes famously evade host immunity using a system of antigenic variation, involving tightly regulated mono-allelic expression and switching of variant surface glycoprotein genes,¹⁷⁹ but this is not conserved in *T. cruzi*. The *T. cruzi* genome contains enormous repetitive arrays of surface protein genes, and there is evidence that some of these gene families or sub-families are reserved for expression in specific life cycle stages.^{2,180} Signatures of strong positive selection in surface gene families¹⁸¹ indicate immune pressure for diversification of antigens. At the population level, simultaneous expression of massively diverse 'decoy' antigens may conceivably prevent T- or B-cell clones specific to any particular epitope from reaching sufficient frequency in infected tissues and/or effector capacity,^{83,112,180,182} but direct evidence for this is lacking.

Very little is known about how variant copy expression may be controlled at the individual cell level, that is amongst amastigotes

and amongst trypomastigotes. Investigating this is difficult, because gene control is mainly post-transcriptional and suitable variant-specific monoclonal antibodies are lacking. Available evidence suggests that within a class of surface proteins, expression in individual parasites is not strictly mono-allelic. For example, trypomastigotes can co-express at least two members of the mucin¹⁸³ and GP85 families.¹⁸² The finding that a specific mucin-associated surface protein (MASP) peptide was only expressed in ~5% of parasites indicates that neither is expression totally promiscuous at the protein level.¹⁸⁴ Mechanisms controlling the expression of parasite surface proteins may therefore vary between gene families or sub-families. *T. cruzi* may also regulate its antigenic repertoire expression between infection phases and between different host cell types. This requires much deeper analysis because currently there are insufficient data to rule out clonal antigenic variation as a mechanism contributing to perpetual immune evasion.

5.2 | Parasite dormancy

Many pathogens use dormancy or metabolic quiescence as an immune evasion strategy.^{185,186} At the population level, *T. cruzi* amastigotes can rapidly decrease their replication rate in response to changes in in vitro culture conditions, but this is a function of a longer G₁ phase rather than exit from the cell cycle.¹⁸⁷ Individual non-replicating amastigotes also occur spontaneously in vitro and are less susceptible to the anti-parasitic drug benznidazole.²¹ The frequency of 4-day replication arrested in vitro amastigotes has been estimated to be approximately 0.1%-6%, depending on the parasite strain.¹⁸⁸ The in vivo relevance of these phenomena remains almost completely unknown and will be hard to establish definitively, not least because neither amastigote DNA/kDNA replication, nor differentiation to constitutively non-replicating trypomastigotes is synchronized.²² Nevertheless, it is reasonable to suspect that slowly replicating or transiently arrested intracellular parasites could have a selective advantage under immunological pressure and play a role in sustaining chronic infections.

5.3 | Cytokine-mediated suppression of type 1 responses

T. cruzi may continue to benefit from the above-mentioned conserved negative-feedback mechanisms that damp down the acute inflammatory response. However, administration of blocking antibodies targeting IL-10 signalling had no discernible effect on chronic *T. cruzi* infections.¹⁷⁴ This is in stark contrast to the well-established role of IL-10 in promoting chronicity of infections with the related parasite *Leishmania* spp.,¹⁸⁹ which predominantly infects professional antigen-presenting cells. Chemical inhibition of the TGF-beta type I receptor significantly alleviated cardiac pathology and function in chronically infected mice, but this was not associated with any change in heart parasite loads.¹⁹⁰ It should be noted that parasite loads in the chronic phase

are often close to the limit of detection, which means that in these types of intervention experiment it is relatively clear when immunity is compromised, but difficult to conclusively demonstrate a significant enhancement of infection control.

Cytokine gene expression in heart tissue from human patients with severe chronic Chagas cardiomyopathy remains strongly polarized to a type 1 profile.¹⁹¹ Type 2 cytokine (IL-4, IL-5, IL-13) expression is reported as undetectable,¹⁹¹ and while it is a feature of some animal models, this is apparently not at the expense of IFN γ production.¹⁷⁰ Interestingly, helminth co-infection is associated with reduced control of *T. cruzi* in a subset of patients, potentially as a result of a modulation of the cytokine balance.¹⁹² Overall, cytokine-mediated suppression of anti-parasitic type 1 inflammation likely influences the host-parasite equilibrium and long-term disease progression, but there is little evidence that it explains *T. cruzi* chronicity.

5.4 | Immunological exhaustion

T-cell exhaustion is a feature of many infectious and non-infectious diseases that involve chronic antigen stimulation and this has been a recent focus of research in the Chagas disease field. Analysis of PBMCs from Chagas patients revealed increased frequencies of CD4⁺ and CD8⁺ T cells expressing exhaustion markers, for example PD-1⁺, CTLA4⁺, or TIM-3⁺.¹⁹³⁻¹⁹⁵ Experimental studies suggest the development of exhaustion characteristics may be promoted by suboptimal B cell,¹⁵² IL-17A¹⁹⁶ or IL-10¹⁵⁶ immune responses. Nevertheless, in chronically infected children and mice, both effector and memory CD8⁺ T cells retain cytotoxic capacity and there is little to no evidence of *functional* exhaustion.^{119,152,174,177,197} Infection chronicity may also promote dysregulation of the Tfh and B-cell compartments, for example, distinct phenotypes and frequencies of these have been noted between symptomatic and asymptomatic *T. cruzi*-infected individuals.^{198,199} Whether these alterations reflect a process of B-cell exhaustion which negatively impacts parasite control remains to be further elucidated. In summary, while deterioration of lymphocyte functional capacity may potentially be associated with progression from asymptomatic to symptomatic disease states, via progressively loosened control of parasite loads, exhaustion does not seem to be a core reason for parasite persistence *per se*.

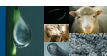
5.5 | Local and hyper-local immune privilege

The realization that long-term *T. cruzi* infections exhibit an unexpectedly high degree of spatio-temporal dynamism⁴ indicates that host responses and evasion mechanisms, including those set out above, need to be studied more intensively at the tissue-specific level. Motile trypomastigotes probably traffic between tissues in both blood and lymph, but there is also evidence that a significant amount of parasite trafficking between tissues may occur inside SLAMF1⁺ myeloid cells, akin to a Trojan horse strategy.²⁰⁰ Consequently,

parasites from privileged reservoir sites, such as the digestive tract, may seed other, less permissive sites such as the heart, resulting in episodic cycles of re-invasion and locally sterilizing host responses (Figure 2).²⁶ Tissue-specific variability in permissiveness is consistent with divergent responses observed in different secondary lymphoid organs.²⁰¹ Moreover, when chronically infected mice are immunosuppressed, the infection relapses first in the GI tract and then disseminates to other organs.²⁹ Host microbiota may also play a role: its composition can be modulated by *T. cruzi* infection,²⁰² but it is not yet known whether this in turn influences anti-parasite immunity in barrier tissues.

To keep up with the parasite, effector cells must be continually deployed to infection foci in many organs. There appears to be no problem with T-cell homing and entry into infected tissues,²⁰³ which is dependent on expression of integrins including VLA-4 and LFA-1,^{174,204} and CXCR3 chemokine receptor signalling.²⁰⁵ After extravasation though, the distinct microenvironment of each organ potentially drives phenotypic changes to infiltrating cells, and in some cases, the effect may be tolerogenic and incompatible with local sterilization. For example, skeletal muscle bulk CTLs recovered from early chronic phase mice produced less IFN γ and had greatly diminished cytotoxic activity compared to splenic CTLs.²⁰⁶ Similar results have been reported for cardiac muscle compared to blood.²⁰³ Intriguingly, splenic CTLs adoptively transferred from one chronically infected mouse to another retained a high IFN γ response phenotype if they migrated to spleen or lung tissue, but lost it if they migrated to skeletal muscle or liver.²⁰⁶ More recently, however, direct *ex vivo* analysis of parasite-specific CTLs without antigen re-stimulation showed cells from chronically infected skeletal muscle tissue had equal or even greater effector capacity (production of IFN γ , TNF- α , granzyme B) than spleen-derived cells.¹⁷⁴ To our knowledge, detailed analysis of CTLs in smooth muscle has yet to be conducted.

There is thus likely to be further compartmentalization of response and evasion dynamics at the intra-organ level, perhaps even down to the hyper-local scale of individual infected cells' microenvironments. For example, the muscular, neuronal and mucosal layers of the GI tract, a key site of *T. cruzi* persistence, have distinct immunological microenvironments that respond differently to *Salmonella* infection.²⁰⁷ Recent work has highlighted differences in the cellular composition of perivascular and parenchymal inflammatory infiltrates in *T. cruzi*-infected skeletal muscle.^{171,178} Large, apparently immunologically invisible parasite nests even occur immediately adjacent to severely inflamed blood vessels, which led these authors to suggest leucocytes might fail to migrate through the parenchyma to infected cells because chemoattractant signalling is too weak in low parasite load settings.¹⁷¹ Immune evasion may also operate at the level of physical interaction between T cells and parasite antigen-presenting cells, for example via manipulation of MHC class I or II expression^{107,208-210} or by parasitism of muscle cells, which are poor activators of NF- κ B upon *T. cruzi* infection⁷⁶ and, in the case of skeletal muscle, do not normally express MHC class I.²¹¹



6 | CONCLUSIONS

From its origins in ancient South American fauna, *T. cruzi* has spread to diverse mammalian orders across the Americas and become a widespread human pathogen. This reflects a remarkable adaptability to evade mammalian immune responses and maintain enzootic, zoonotic and anthroponotic transmission cycles. As we have set out, this involves sophisticated molecular mechanisms that allow *T. cruzi* to resist innate responses so that in the early stages of infection parasite loads are high and widely disseminated in blood and solid organs. The adaptive immune response, principally effected by CTLs and antibodies, is able to eliminate ~99% of the parasites. The infection then transitions to a permanent chronic phase in which parasites replicate, mainly within muscle cells in a small number of privileged tissues, in a dynamic equilibrium with host responses. The available evidence supports the existence of a complex set of molecular and cellular mediators that firstly, prevent complete sterile clearance at the acute to chronic transition and, secondly, ensure perpetual parasite persistence during the chronic phase. These include parasite-intrinsic evasion mechanisms, direct and indirect manipulation of host responses, and host-intrinsic deactivating feedback loops. Further complexity arises from *T. cruzi*'s cycling between intra- and extracellular parasite forms and its trafficking between different organs, tissues and cells, each with specific immunological microenvironments of variable permissiveness.

Important advances in fundamental aspects of tissue level immunity have yet to be investigated in detail in the Chagas disease field, for example, defining relative contributions of tissue-resident and inflammatory myeloid cells, innate lymphoid cell populations, tissue-resident memory T cells and neuro-immune interactions. Further progress in understanding *T. cruzi*-host interactions and how they shape Chagas disease pathogenesis is also likely to come from more intensive research at the tissue-specific and even single cell scale. Some key questions include the following: (a) why do some infected people remain asymptomatic carriers while others progress to life-threatening disease states? (b) Does active infection support concomitant immunity to second infections? (c) Do drug-cured patients have protective immunity to re-infection? (d) Can vaccines be developed that provide sterile protection? (e) To what degree are the proposed mechanisms of immune evasion actually enabling parasite persistence in vivo in different tissues? (f) How do host-parasite interactions promote or limit *in utero* transmission? The difficulties of answering these questions and addressing the wider challenges in Chagas disease biomedicine are great; however, the massive unmet need for better treatments, prophylaxis and diagnostics requires us to overcome them.

ACKNOWLEDGEMENTS

The authors' research is supported by UK Medical Research Council (MRC) grant MR/R021430/1 (MDL), MRC doctoral studentship MR/N013638/1 (AIW) and Hull York Medical School (DP-M).

CONFLICT OF INTEREST

None.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/pim.12786>.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analysed during the current study.

ORCID

Damián Pérez-Mazliah  <https://orcid.org/0000-0002-2156-2585>

Michael D. Lewis  <https://orcid.org/0000-0001-9540-3280>

REFERENCES

1. Brenière SF, Waleckx E, Barnabé C. Over six thousand *Trypanosoma cruzi* strains classified into discrete typing units (DTUs): attempt at an inventory. *PLoS Negl Trop Dis*. 2016;10(8):e0004792.
2. Buscaglia CA, Campo VA, Frasch AC, Di Noia JM. *Trypanosoma cruzi* surface mucins: host-dependent coat diversity. *Nat Rev Microbiol*. 2006;4(3):229-236.
3. Barisón MJ, Rapado LN, Merino EF, et al. Metabolomic profiling reveals a finely tuned, starvation-induced metabolic switch in *Trypanosoma cruzi* epimastigotes. *J Biol Chem*. 2017;292(21):8964-8977.
4. Lewis MD, Francisco AF, Taylor MC, et al. Bioluminescence imaging of chronic *Trypanosoma cruzi* infections reveals tissue-specific parasite dynamics and heart disease in the absence of locally persistent infection. *Cell Microbiol*. 2014;16(9):1285-1300.
5. Hyland KV, Asfaw SH, Olson CL, Daniels MD, Engman DM. Bioluminescent imaging of *Trypanosoma cruzi* infection. *Int J Parasitol*. 2008;38(12):1391-1400.
6. Zhang L, Tarleton RL. Parasite persistence correlates with disease severity and localization in chronic Chagas' disease. *J Infect Dis*. 1999;180(2):480-486.
7. Francisco AF, Lewis MD, Jayawardhana S, Taylor MC, Chatelain E, Kelly JM. The limited ability of posaconazole to cure both acute and chronic *Trypanosoma cruzi* infections revealed by highly sensitive in vivo imaging. *Antimicrob Agents Chemother*. 2015;59(8):4653-4661.
8. Lenzi HL, Oliveira DN, Lima MT, Gattass CR. *Trypanosoma cruzi*: paninfectivity of CL strain during murine acute infection. *Exp Parasitol*. 1996;84(1):16-27.
9. Guarner J, Bartlett J, Zaki SR, Colley DG, Grijalva MJ, Powell MR. Mouse model for Chagas disease: immunohistochemical distribution of different stages of *Trypanosoma cruzi* in tissues throughout infection. *Am J Trop Med Hyg*. 2001;65(2):152-158.
10. Morocoima A, Rodríguez M, Herrera L, Urdaneta-Morales S. *Trypanosoma cruzi*: experimental parasitism of bone and cartilage. *Parasitol Res*. 2006;99(6):663-668.
11. Calabrese KS, Lagrange PH, da Costa SC. *Trypanosoma cruzi*: histopathology of endocrine system in immunocompromised mice. *Int J Exp Pathol*. 1994;75(6):453-462.
12. Postan M, Dvorak JA, McDaniel JP. Studies of *Trypanosoma cruzi* clones in inbred mice: I. A comparison of the course of infection of C3H/HEN- mice with two clones isolated from a common source. *Am J Trop Med Hyg*. 1983;32(3):497-506.
13. Melo R, Brener Z. Tissue tropism of different *Trypanosoma cruzi* strains. *J Parasitol*. 1978;64(3):475-482.
14. Buckner FS, Wilson AJ, Van Voorhis WC. Detection of live *Trypanosoma cruzi* in tissues of infected mice by using histochemical stain for β -Galactosidase. *Infect Immun*. 1999;67(1):403-409.
15. Camandaroba E, Thé TS, Pessina DH, Andrade SG. *Trypanosoma cruzi*: clones isolated from the Colombian strain, reproduce the

- parental strain characteristics, with ubiquitous histotropism. *Int J Exp Pathol*. 2006;87(3):209-217.
16. Gómez-Hernández C, Rezende-Oliveira K, Nascentes GAN, et al. Molecular characterization of *Trypanosoma cruzi* Mexican strains and their behavior in the mouse experimental model. *Rev Soc Bras Med Trop*. 2011;44:684-690.
 17. Almeida-de-Faria M, Freymüller E, Colli W, Alves MJM. *Trypanosoma cruzi*: characterization of an intracellular epimastigote-like form. *Exp Parasitol*. 1999;92(4):263-274.
 18. Tyler KM, Engman DM. The life cycle of *Trypanosoma cruzi* revisited. *Int J Parasitol*. 2001;31(5-6):472-480.
 19. Kessler RL, Contreras VT, Marlière NP, et al. Recently differentiated epimastigotes from *Trypanosoma cruzi* are infective to the mammalian host. *Mol Microbiol*. 2017;104(5):712-736.
 20. Kurup SP, Tarleton RL. The *Trypanosoma cruzi* flagellum is discarded via asymmetric cell division following invasion and provides early targets for protective CD8⁺ T cells. *Cell Host Microbe*. 2014;16(4):439-449.
 21. Sánchez-Valdéz FJ, Padilla A, Wang W, Orr D, Tarleton RL. Spontaneous dormancy protects *Trypanosoma cruzi* during extended drug exposure. *eLife*. 2018;7:e34039.
 22. Taylor MC, Ward A, Olmo F, et al. Intracellular DNA replication and differentiation of *Trypanosoma cruzi* is asynchronous within individual host cells in vivo at all stages of infection. *PLoS Negl Trop Dis*. 2020;14(3):e0008007.
 23. Sabino EC, Ribeiro AL, Salemi VMC, et al. Ten-year incidence of chagas cardiomyopathy among asymptomatic *Trypanosoma cruzi*-seropositive former blood donors. *Circulation*. 2013;127(10):1105-1115.
 24. Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. *Lancet*. 2010;375(9723):1388-1402.
 25. Chatelain E, Konar N. Translational challenges of animal models in Chagas disease drug development: a review. *Drug Des Devel Ther*. 2015;9:4807.
 26. Lewis MD, Kelly JM. Putting infection dynamics at the heart of Chagas disease. *Trends Parasitol*. 2016;32(11):899-911.
 27. Andriani G, Chessler A-DC, Courtemanche G, Burleigh BA, Rodriguez A. Activity in vivo of anti-*Trypanosoma cruzi* compounds selected from a high throughput screening. *PLoS Negl Trop Dis*. 2011;5(8):e1298.
 28. Henriques C, Henriques-Pons A, Meuser-Batista M, Ribeiro AS, de Souza W. In vivo imaging of mice infected with bioluminescent *Trypanosoma cruzi* unveils novel sites of infection. *Parasit Vectors*. 2014;7(1):1-15.
 29. Lewis MD, Francisco AF, Taylor MC, Jayawardhana S, Kelly JM. Host and parasite genetics shape a link between *Trypanosoma cruzi* infection dynamics and chronic cardiomyopathy. *Cell Microbiol*. 2016;18(10):1429-1443.
 30. Silberstein E, Serna C, Fragoso SP, Nagarkatti R, Debrabant A. A novel nanoluciferase-based system to monitor *Trypanosoma cruzi* infection in mice by bioluminescence imaging. *PLoS One*. 2018;13(4):e0195879.
 31. Silva-dos-Santos D, Barreto-de-Albuquerque J, Guerra B, et al. Unraveling Chagas disease transmission through the oral route: Gateways to *Trypanosoma cruzi* infection and target tissues. *PLoS Negl Trop Dis*. 2017;11(4):e0005507.
 32. Ferreira BL, Orikaza CM, Cordero EM, Mortara RA. *Trypanosoma cruzi*: single cell live imaging inside infected tissues. *Cell Microbiol*. 2016;18(6):779-783.
 33. Canavaci AMC, Bustamante JM, Padilla AM, et al. In vitro and in vivo high-throughput assays for the testing of anti-*Trypanosoma cruzi* compounds. *PLoS Negl Trop Dis*. 2010;4(7):e740.
 34. Costa FC, Francisco AF, Jayawardhana S, et al. Expanding the toolbox for *Trypanosoma cruzi*: A parasite line incorporating a bioluminescence-fluorescence dual reporter and streamlined CRISPR/Cas9 functionality for rapid in vivo localisation and phenotyping. *PLoS Negl Trop Dis*. 2018;12(4):e0006388.
 35. Mann GS, Francisco AF, Jayawardhana S, et al. Drug-cured experimental *Trypanosoma cruzi* infections confer long-lasting and cross-strain protection. *PLoS Negl Trop Dis*. 2020;14(4):e0007717.
 36. Fernandes MC, Andrews NW. Host cell invasion by *Trypanosoma cruzi*: a unique strategy that promotes persistence. *FEMS Microbiol Rev*. 2012;36(3):734-747.
 37. Machado FS, Dutra WO, Esper L, et al. Current understanding of immunity to *Trypanosoma cruzi* infection and pathogenesis of Chagas disease. *Semin Immunopathol*. 2012;34(6):753-770.
 38. Lewis MD, Francisco AF, Jayawardhana S, Langston H, Taylor MC, Kelly JM. Imaging the development of chronic Chagas disease after oral transmission. *Sci Rep*. 2018;8(1):11292.
 39. Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. *Clin Infect Dis*. 2012;54(6):845-852.
 40. Hoft DF, Farrar PL, Kratz-Owens K, Shaffer D. Gastric invasion by *Trypanosoma cruzi* and induction of protective mucosal immune responses. *Infect Immun*. 1996;64(9):3800-3810.
 41. Giddings OK, Eickhoff CS, Smith TJ, Bryant LA, Hoft DF. Anatomical route of invasion and protective mucosal immunity in *Trypanosoma cruzi* conjunctival infection. *Infect Immun*. 2006;74(10):5549-5560.
 42. Andrade SG, Magalhães JB. Biodemes and zymodemes of *Trypanosoma cruzi* strains: correlations with clinical data and experimental pathology. *Rev Soc Bras Med Tro*. 1997;30:27-35.
 43. Taliaferro WH, Tulo P. Connective tissue reactions in normal and immunized mice to a reticulotropic strain of *Trypanosoma cruzi*. *J Infect Dis*. 1955;96(3):199-226.
 44. Zeledón R, Ponce C. Neurotropism in Costa Rican strains of *Trypanosoma cruzi*. *J Parasitol*. 1972;58(1):180-181.
 45. Córdova E, Maiolo E, Corti M, Orduña T. Neurological manifestations of Chagas' disease. *Neurol Res*. 2010;32(3):238-244.
 46. Tarleton RL. CD8⁺ T cells in *Trypanosoma cruzi* infection. *Semin Immunopathol*. 2015;37(3):233-238.
 47. Padilla AM, Simpson LJ, Tarleton RL. Insufficient TLR activation contributes to the slow development of CD8⁺ T cell responses in *Trypanosoma cruzi* infection. *J Immunol*. 2009;183(2):1245-1252.
 48. Chessler A-DC, Unnikrishnan M, Bei AK, Daily JP, Burleigh BA. *Trypanosoma cruzi* triggers an early type I IFN response in vivo at the site of intradermal infection. *J Immunol*. 2009;182(4):2288-2296.
 49. Kayama H, Koga R, Atarashi K, et al. NFATc1 mediates toll-like receptor-independent innate immune responses during *Trypanosoma cruzi* infection. *PLoS Pathog*. 2009;5(7):e1000514.
 50. Reina-San-Martín B, Degraeve W, Rougeot C, et al. A B-cell mitogen from a pathogenic trypanosome is a eukaryotic proline racemase. *Nat Med*. 2000;6(8):890-897.
 51. Gao W, Wortis HH, Pereira MA. The *Trypanosoma cruzi* trans-sialidase is a T cell-independent B cell mitogen and an inducer of non-specific Ig secretion. *Int Immunol*. 2002;14(3):299-308.
 52. Bermejo DA, Amezcua Vesely MC, Khan M, et al. *Trypanosoma cruzi* infection induces a massive extrafollicular and follicular splenic B-cell response which is a high source of non-parasite-specific antibodies. *Immunology*. 2011;132(1):123-133.
 53. Campos MAS, Almeida IC, Takeuchi O, et al. Activation of toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *J Immunol*. 2001;167(1):416-423.
 54. Bafica A, Santiago HC, Goldszmid R, Ropert C, Gazzinelli RT, Sher A. Cutting edge: TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in *Trypanosoma cruzi* infection. *J Immunol*. 2006;177(6):3515-3519.
 55. Koga R, Hamano S, Kuwata H, et al. TLR-dependent induction of IFN-beta mediates host defense against *Trypanosoma cruzi*. *J Immunol*. 2006;177(10):7059-7066.
 56. Oliveira A-C, de Alencar BC, Tzelepis F, et al. Impaired innate immunity in Tlr4^{-/-} mice but preserved CD8⁺ T cell responses

- against *Trypanosoma cruzi* in Tlr4-, Tlr2-, Tlr9- or Myd88-deficient mice. *PLoS Pathog.* 2010;6(4):e1000870.
57. Oliveira A-C, Peixoto JR, de Arruda LB, et al. Expression of functional TLR4 confers proinflammatory responsiveness to *Trypanosoma cruzi* glycoinositolphospholipids and higher resistance to infection with *T. cruzi*. *J Immunol.* 2004;173(9):5688.
 58. Caetano BC, Carmo BB, Melo MB, et al. Requirement of UNC93B1 reveals a critical role for TLR7 in host resistance to primary infection with *Trypanosoma cruzi*. *J Immunol.* 2011;187(4):1903-1911.
 59. Pineda MA, Cuervo H, Fresno M, Soto M, Bonay P. Lack of galectin-3 prevents cardiac fibrosis and effective immune responses in a murine model of *Trypanosoma cruzi* infection. *J Infect Dis.* 2015;212(7):1160-1171.
 60. Benatar AF, García GA, Bua J, et al. Galectin-1 prevents infection and damage induced by *Trypanosoma cruzi* on cardiac cells. *PLoS Negl Trop Dis.* 2015;9(10):e0004148.
 61. Shi W, Xue C, Su XZ, Lu F. The roles of galectins in parasitic infections. *Acta Trop.* 2018;177:97-104.
 62. Silva GK, Gutierrez FRS, Guedes PMM, et al. Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. *J Immunol.* 2010;184(3):1148-1152.
 63. Gonçalves VM, Matteucci KC, Buzzo CL, et al. NLRP3 controls *Trypanosoma cruzi* infection through a Caspase-1-dependent IL-1R-independent NO production. *PLoS Negl Trop Dis.* 2013;7(10):e2469.
 64. Paroli AF, Gonzalez PV, Díaz-Luján C, et al. NLRP3 inflammasome and caspase-1/11 pathway orchestrate different outcomes in the host protection against *Trypanosoma cruzi* acute infection. *Front Immunol.* 2018;9:913.
 65. Silva GK, Costa RS, Silveira TN, et al. Apoptosis-associated speck-like protein containing a caspase recruitment domain inflammasomes mediate IL-1 β response and host resistance to *Trypanosoma cruzi* infection. *J Immunol.* 2013;191(6):3373-3383.
 66. Li Y, Shah-Simpson S, Okrah K, et al. Transcriptome remodeling in *Trypanosoma cruzi* and human cells during intracellular infection. *PLoS Pathog.* 2016;12(4):e1005511.
 67. Bayer-Santos E, Gentil LG, Cordero EM, Correa PR, Silveira JF. Regulatory elements in the 3' untranslated region of the GP82 glycoprotein are responsible for its stage-specific expression in *Trypanosoma cruzi* metacyclic trypomastigotes. *Acta Trop.* 2012;123(3):230-233.
 68. de Pablos Torró LM, Retana Moreira L, Osuna A. Extracellular vesicles in Chagas disease: a new passenger for an old disease. *Front Microbiol.* 2018;9:1190.
 69. Ribeiro KS, Vasconcellos CI, Soares RP, et al. Proteomic analysis reveals different composition of extracellular vesicles released by two *Trypanosoma cruzi* strains associated with their distinct interaction with host cells. *J Extracell Vesicles.* 2018;7(1):1463779.
 70. Caeiro LD, Alba-Soto CD, Rizzi M, et al. The protein family TcTASV-C is a novel *Trypanosoma cruzi* virulence factor secreted in extracellular vesicles by trypomastigotes and highly expressed in bloodstream forms. *PLoS Negl Trop Dis.* 2018;12(5):e0006475.
 71. Choudhuri S, Garg NJ. PARP1-cGAS-NF- κ B pathway of proinflammatory macrophage activation by extracellular vesicles released during *Trypanosoma cruzi* infection and Chagas disease. *PLoS Pathog.* 2020;16(4):e1008474.
 72. Cribb P, Perdomo V, Alonso VL, et al. *Trypanosoma cruzi* High Mobility Group B (TcHMGB) can act as an inflammatory mediator on mammalian cells. *PLoS Negl Trop Dis.* 2017;11(2):e0005350.
 73. Huang H, Petkova SB, Cohen AW, et al. Activation of transcription factors AP-1 and NF- κ B in murine chagasic myocarditis. *Infect Immun.* 2003;71(5):2859-2867.
 74. Chessler A-DC, Ferreira LRP, Chang T-H, Fitzgerald KA, Burleigh BA. A novel IFN regulatory factor 3-dependent pathway activated by trypanosomes triggers IFN- β in macrophages and fibroblasts. *J Immunol.* 2008;181(11):7917-7924.
 75. Dias WB, Fajardo FD, Graça-Souza AV, et al. Endothelial cell signalling induced by trans-sialidase from *Trypanosoma cruzi*. *Cell Microbiol.* 2008;10(1):88-99.
 76. Hall BS, Tam W, Sen R, Pereira MEA. Cell-specific activation of nuclear factor- κ B by the parasite *Trypanosoma cruzi* promotes resistance to intracellular infection. *Mol Biol Cell.* 2000;11(1):153-160.
 77. Michailowsky V, Silva NM, Rocha CD, Vieira LQ, Lannes-Vieira J, Gazzinelli RT. Pivotal role of interleukin-12 and interferon- γ axis in controlling tissue parasitism and inflammation in the heart and central nervous system during *Trypanosoma cruzi* infection. *Am J Pathol.* 2001;159(5):1723-1733.
 78. Castaños-Velez E, Maerlan S, Osorio LM, et al. *Trypanosoma cruzi* infection in tumor necrosis factor receptor p55-deficient mice. *Infect Immun.* 1998;66(6):2960-2968.
 79. McGovern KE, Wilson EH. Role of chemokines and trafficking of immune cells in parasitic infections. *Curr Immunol Rev.* 2013;9(3):157-168.
 80. Scharfstein J. Subverting bradykinin-evoked inflammation by co-opting the contact system: lessons from survival strategies of *Trypanosoma cruzi*. *Curr Opin Hematol.* 2018;25(5):347-357.
 81. Nascimento CR, Andrade D, Carvalho-Pinto CE, et al. Mast cell coupling to the Kallikrein-Kinin system fuels intracardiac parasitism and Worsens Heart Pathology in experimental Chagas disease. *Front Immunol.* 2017;8:840.
 82. Casassa AF, Vanrell MC, Colombo MI, Gottlieb RA, Romano PS. Autophagy plays a protective role against *Trypanosoma cruzi* infection in mice. *Virulence.* 2019;10(1):151-165.
 83. Cardoso MS, Reis-Cunha JL, Bartholomeu DC. Evasion of the immune response by *Trypanosoma cruzi* during acute infection. *Front Immunol.* 2016;6:659.
 84. Lieke T, Graefe SEB, Klauenberg U, Fleischer B, Jacobs T. NK cells contribute to the control of *Trypanosoma cruzi* infection by killing free parasites by perforin-independent mechanisms. *Infect Immun.* 2004;72(12):6817-6825.
 85. Baez NS, Cerbán F, Savid-Frontera C, et al. Thymic expression of IL-4 and IL-15 after systemic inflammatory or infectious Th1 disease processes induce the acquisition of "innate" characteristics during CD8+ T cell development. *PLoS Pathog.* 2019;15(1):e1007456.
 86. Mesías AC, Garg NJ, Zago MP. Redox balance keepers and possible cell functions managed by redox homeostasis in *Trypanosoma cruzi*. *Front Cell Infect Microbiol.* 2019;9:435.
 87. Arantes RME, Marche HHF, Bahia MT, Cunha FQ, Rossi MA, Silva JS. Interferon- γ -induced nitric oxide causes intrinsic intestinal denervation in *Trypanosoma cruzi*-infected mice. *Am J Pathol.* 2004;164(4):1361-1368.
 88. Paiva CN, Feijó DF, Dutra FF, et al. Oxidative stress fuels *Trypanosoma cruzi* infection in mice. *J Clin Invest.* 2012;122(7):2531-2542.
 89. Vespa GN, Cunha FQ, Silva JS. Nitric oxide is involved in control of *Trypanosoma cruzi*-induced parasitemia and directly kills the parasite in vitro. *Infect Immun.* 1994;62(11):5177-5182.
 90. Hölscher C, Köhler G, Müller U, Mossman H, Schaub GA, Brombacher F. Defective nitric oxide effector functions lead to extreme susceptibility of *Trypanosoma cruzi*-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. *Infect Immun.* 1998;66(3):1208-1215.
 91. Cummings KL, Tarleton RL. Inducible nitric oxide synthase is not essential for control of *Trypanosoma cruzi* infection in mice. *Infect Immun.* 2004;72(7):4081-4089.
 92. Doyle PS, Zhou YM, Engel JC, McKerrow JH. A cysteine protease inhibitor cures Chagas disease in an immunodeficient-mouse model of infection. *Antimicrob Agents Chemother.* 2007;51(11):3932-3939.
 93. Hoft DF, Schnapp AR, Eickhoff CS, Roodman ST. Involvement of CD4+ Th1 cells in systemic immunity protective against primary

- and secondary challenges with *Trypanosoma cruzi*. *Infect Immun*. 2000;68(1):197-204.
94. Trischmann T, Tanowitz H, Wittner M, Bloom B. *Trypanosoma cruzi*: role of the immune response in the natural resistance of inbred strains of mice. *Exp Parasitol*. 1978;45(2):160-168.
 95. Francolino SS, Antunes AF, Talice R, et al. New evidence of spontaneous cure in human Chagas' disease. *Rev Soc Bras Med Trop*. 2003;36:103-107.
 96. Acevedo GR, Girard MC, Gómez KA. The unsolved jigsaw puzzle of the immune response in Chagas disease. *Front Immunol*. 2018;9:1929.
 97. Acosta Rodríguez EV, Araujo Furlan CL, Fiocca Vernengo F, Montes CL, Gruppi A. Understanding CD8+ T cell immunity to *Trypanosoma cruzi* and how to improve it. *Trends Parasitol*. 2019;35(11):899-917.
 98. Kumar S, Tarleton RL. The relative contribution of antibody production and CD8+ T cell function to immune control of *Trypanosoma cruzi*. *Parasite Immunol*. 1998;20(5):207-216.
 99. Tzelepis F, Persechini PM, Rodrigues MM. Modulation of CD4+ T cell-dependent specific cytotoxic CD8+ T cells differentiation and proliferation by the timing of increase in the pathogen load. *PLoS One*. 2007;2(4):e393.
 100. Tarleton R, Koller B, Latour A, Postan M. Susceptibility of β 2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. *Nature*. 1992;356(6367):338-340.
 101. Tarleton RL, Sun J, Zhang L, Postan M. Depletion of T-cell subpopulations results in exacerbation of myocarditis and parasitism in experimental Chagas' disease. *Infect Immun*. 1994;62(5):1820-1829.
 102. Cardillo F, Postol E, Nihei J, Aroeira LS, Nomizo A, Mengel J. B cells modulate T cells so as to favour T helper type 1 and CD8+ T cell responses in the acute phase of *Trypanosoma cruzi* infection. *Immunology*. 2007;122(4):584-595.
 103. Dominguez MR, Ersching J, Lemos R, et al. Re-circulation of lymphocytes mediated by sphingosine-1-phosphate receptor-1 contributes to resistance against experimental infection with the protozoan parasite *Trypanosoma cruzi*. *Vaccine*. 2012;30(18):2882-2891.
 104. Bixby LM, Tarleton RL. Stable CD8+ T cell memory during persistent *Trypanosoma cruzi* infection. *J Immunol*. 2008;181(4):2644-2650.
 105. Mucci J, Hidalgo A, Mocetti E, Argibay PF, Leguizamón MS, Campetella O. Thymocyte depletion in *Trypanosoma cruzi* infection is mediated by trans-sialidase-induced apoptosis on nurse cells complex. *Proc Natl Acad Sci USA*. 2002;99(6):3896-3901.
 106. Ersching J, Basso AS, Kalich VLG, Bortoluci KR, Rodrigues MM. A human trypanosome suppresses CD8+ T cell priming by dendritic cells through the induction of immune regulatory CD4+ Foxp3+ T cells. *PLoS Pathog*. 2016;12(6):e1005698.
 107. Overtvelt LV, Andrieu M, Verhasselt V, et al. *Trypanosoma cruzi* down-regulates lipopolysaccharide-induced MHC class I on human dendritic cells and impairs antigen presentation to specific CD8+ T lymphocytes. *Int Immunol*. 2002;14(10):1135-1144.
 108. Poncini CV, Soto CDA, Batalla E, Solana ME, González Cappa SM. *Trypanosoma cruzi* induces regulatory dendritic cells in vitro. *Infect Immun*. 2008;76(6):2633-2641.
 109. Poncini CV, Ilarregui JM, Batalla EI, et al. *Trypanosoma cruzi* infection imparts a regulatory program in dendritic cells and T cells via galectin-1-dependent mechanisms. *J Immunol*. 2015;195(7):3311-3324.
 110. Martin DL, Weatherly DB, Laucella SA, et al. CD8+ T-cell responses to *Trypanosoma cruzi* are highly focused on strain-variant trans-sialidase epitopes. *PLoS Pathog*. 2006;2(8):e77.
 111. Tzelepis F, de Alencar BC, Penido ML, et al. Infection with *Trypanosoma cruzi* restricts the repertoire of parasite-specific CD8+ T cells leading to immunodominance. *J Immunol*. 2008;180(3):1737-1748.
 112. Rosenberg CS, Zhang W, Bustamante JM, Tarleton RL. Long-term immunity to *Trypanosoma cruzi* in the absence of immunodominant trans-sialidase-specific CD8+ T cells. *Infect Immun*. 2016;84(9):2627-2638.
 113. Alvarez MG, Postan M, Weatherly DB, et al. HLA Class I-T cell epitopes from trans-sialidase proteins reveal functionally distinct subsets of CD8+ T cells in chronic Chagas disease. *PLoS Negl Trop Dis*. 2008;2(9):e288.
 114. Pinazo M-J, Espinosa G, Cortes-Lletget C, et al. Immunosuppression and Chagas disease: a management challenge. *PLoS Negl Trop Dis*. 2013;7(1):e1965.
 115. Tarleton RL, Grusby MJ, Postan M, Glimcher LH. *Trypanosoma cruzi* infection in MHC-deficient mice: further evidence for the role of both class I- and class II-restricted T cells in immune resistance and disease. *Int Immunol*. 1996;8(1):13-22.
 116. Padilla A, Xu D, Martin D, Tarleton R. Limited role for CD4+ T-cell help in the initial priming of *Trypanosoma cruzi*-specific CD8+ T cells. *Infect Immun*. 2007;75(1):231-235.
 117. Gomes JAS, Bahia-Oliveira LMG, Rocha MOC, Martins-Filho OA, Gazzinelli G, Correa-Oliveira R. Evidence that development of severe cardiomyopathy in human Chagas' disease is due to a Th1-specific immune response. *Infect Immun*. 2003;71(3):1185-1193.
 118. Guo S, Cobb D, Smeltz RB. T-bet inhibits the in vivo differentiation of parasite-specific CD4+ Th17 cells in a T cell-intrinsic manner. *J Immunol*. 2009;182(10):6179-6186.
 119. Albareda MC, De Rissio AM, Tomas G, et al. Polyfunctional T Cell responses in children in early stages of chronic *Trypanosoma cruzi* infection contrast with monofunctional responses of long-term infected adults. *PLoS Negl Trop Dis*. 2013;7(12):e2575.
 120. Bonney KM, Taylor JM, Daniels MD, Epting CL, Engman DM. Heat-killed *Trypanosoma cruzi* induces acute cardiac damage and polyanitigenic autoimmunity. *PLoS One*. 2011;6(1):e14571.
 121. Santamaría MH, Corral RS. Osteopontin-dependent regulation of Th1 and Th17 cytokine responses in *Trypanosoma cruzi*-infected C57BL/6 mice. *Cytokine*. 2013;61(2):491-498.
 122. Guedes PMM, Gutierrez FRS, Silva GK, et al. Deficient regulatory T cell activity and low frequency of IL-17-producing T cells correlate with the extent of cardiomyopathy in human Chagas' disease. *PLoS Negl Trop Dis*. 2012;6(4):e1630.
 123. Díaz PR, Mucci J, Meira MA, et al. *Trypanosoma cruzi* trans-sialidase prevents elicitation of Th1 cell response via interleukin 10 and downregulates Th1 effector cells. *Infect Immun*. 2015;83(5):2099-2108.
 124. Bryan MA, Guyach SE, Norris KA. Specific humoral immunity versus polyclonal B cell activation in *Trypanosoma cruzi* infection of susceptible and resistant mice. *PLoS Negl Trop Dis*. 2010;4(7):e733.
 125. Chavele K-M, Merry E, Ehrenstein MR. Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. *J Immunol*. 2015;194(6):2482-2485.
 126. Eto D, Lao C, DiToro D, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. *PLoS One*. 2011;6(3):e17739.
 127. Gao W, Pereira MA. Interleukin-6 is required for parasite specific response and host resistance to *Trypanosoma cruzi*. *Int J Parasitol*. 2002;32:167-170.
 128. Bermejo DA, Amezcua-Vesely MC, Montes CL, et al. BAFF mediates splenic B cell response and antibody production in experimental chagas disease. *PLoS Negl Trop Dis*. 2010;4(5):e679.
 129. Bonney KM, Engman DM. Autoimmune pathogenesis of Chagas heart disease: looking back, looking ahead. *Am J Pathol*. 2015;185(6):1537-1547.
 130. Hyland KV, Leon JS, Daniels MD, et al. Modulation of autoimmunity by treatment of an infectious disease. *Infect Immun*. 2007;75(7):3641-3650.

131. Viotti R, Alarcón de Noya B, Araujo-Jorge T, et al. Towards a paradigm shift in the treatment of chronic Chagas disease. *Antimicrob Agents Chemother*. 2014;58(2):635-639.
132. Tarleton RL. Chagas disease: a role for autoimmunity? *Trends Parasitol*. 2003;19(10):447-451.
133. Minoprio P, Burlen O, Pereira P, et al. Most B cells in acute *Trypanosoma cruzi* infection lack parasite specificity. *Scand J Immunol*. 1988;28:553-561.
134. Acosta Rodríguez EV, Zuniga EI, Montes CL, et al. *Trypanosoma cruzi* infection beats the B-cell compartment favouring parasite establishment: can we strike first? *Scand J Immunol*. 2007;66(2-3):137-142.
135. Bryan MA, Norris KA. Genetic immunization converts the *Trypanosoma cruzi* B-cell mitogen proline racemase to an effective immunogen. *Infect Immun*. 2010;78(2):810-822.
136. Bermejo DA, Jackson SW, Gorosito-Serran M, et al. *Trypanosoma cruzi* trans-sialidase initiates a program independent of the transcription factors ROR γ t and Ahr that leads to IL-17 production by activated B cells. *Nat Immunol*. 2013;14(5):514-522.
137. Fares RCG, Correa-Oliveira R, de Arajo FF, et al. Identification of phenotypic markers of B cells from patients with Chagas disease. *Parasite Immunol*. 2013;35(7-8):214-223.
138. Passos LSA, Magalhães LMD, Soares RP, et al. Activation of human CD11b+ B1 B-Cells by *Trypanosoma cruzi*-derived proteins is associated with protective immune response in human Chagas disease. *Front Immunol*. 2019;9:3015.
139. Zuniga E, Acosta-Rodriguez E, Merino MC, Montes C, Gruppi A. Depletion of immature B cells during *Trypanosoma cruzi* infection: involvement of myeloid cells and the cyclooxygenase pathway. *Eur J Immunol*. 2005;35(6):1849-1858.
140. Dotiwala F, Mulik S, Polidoro RB, et al. Killer lymphocytes use granulysin, perforin and granzymes to kill intracellular parasites. *Nat Med*. 2016;22(2):210.
141. de Alencar BCG, Persechini PM, Haolla FA, et al. Perforin and gamma interferon expression are required for CD4+ and CD8+ T-Cell-dependent protective immunity against a human parasite, *Trypanosoma cruzi*, elicited by heterologous plasmid DNA prime-recombinant adenovirus 5 boost vaccination. *Infect Immun*. 2009;77(10):4383-4395.
142. Müller U, Sobek V, Balkow S, et al. Concerted action of perforin and granzymes is critical for the elimination of *Trypanosoma cruzi* from mouse tissues, but prevention of early host death is in addition dependent on the FasL/Fas pathway. *Eur J Immunol*. 2003;33(1):70-78.
143. Silverio JC, De-Oliveira-Pinto LM, Da Silva AA, De Oliveira GM, Lannes-Vieira J. Perforin-expressing cytotoxic cells contribute to chronic cardiomyopathy in *Trypanosoma cruzi* infection. *Int J Exp Pathol*. 2010;91(1):72-86.
144. Leguizamon MS, Campetella O, Russomando G, Almiron M, Guillen I. Antibodies inhibiting *Trypanosoma cruzi* trans-sialidase activity in sera from human infections. *J Infect Dis*. 1994;170(6):1570-1574.
145. Leguizamon MS, Campetella OE, Cappa SMG, Frasc ACC. Mice infected with *Trypanosoma cruzi* produce antibodies against the enzymatic domain of trans-sialidase that inhibit its activity. *Infect Immun*. 1994;62(8):3441-3446.
146. Pereira-Chioccola VL, Schenkman S, Kloetzel JK. Sera from chronic chagasic patients and rodents infected with *Trypanosoma cruzi* inhibit trans-sialidase by recognizing its amino-terminal and catalytic domain. *Infect Immun*. 1994;62(7):2973-2978.
147. Krettl AU, Brener Z. Resistance against *Trypanosoma cruzi* associated to anti-living trypomastigote antibodies. *J Immunol*. 1982;128:2009-2012.
148. Sardinha LR, Mosca T, Elias RM, et al. The liver plays a major role in clearance and destruction of blood trypomastigotes in *Trypanosoma cruzi* chronically infected mice. *PLoS Negl Trop Dis*. 2010;4(1):e578.
149. Umekita LF, Mota I. How are antibodies involved in the protective mechanism of susceptible mice infected with *T. cruzi*? *Braz J Med Biol Res*. 2000;33:253-258.
150. Fiocca Vernengo F, Beccaria CG, Araujo Furlan CL, et al. CD8+ T cell immunity is compromised by anti-CD20 treatment and rescued by interleukin-17A. *mBio*. 2020;11(3):e00447-20.
151. Gorosito Serrán M, Tosello Boari J, Fiocca Vernengo F, et al. Unconventional pro-inflammatory CD4+ T cell response in B Cell-deficient mice infected with *Trypanosoma cruzi*. *Front Immunol*. 2017;8:1548.
152. Sullivan NL, Eickhoff CS, Sagartz J, Hoft DF. Deficiency of antigen-specific B cells results in decreased *Trypanosoma cruzi* systemic but not mucosal immunity due to CD8 T Cell exhaustion. *J Immunol*. 2015;194(4):1806-1818.
153. Rassi A, Marin-Neto JA. Chagas disease. *Lancet*. 2010;375:1388-1402.
154. Hölscher C, Mohrs M, Dai WJ, et al. Tumor necrosis factor alpha-mediated toxic shock in *Trypanosoma cruzi*-infected interleukin 10-deficient mice. *Infect Immun*. 2000;68(7):4075-4083.
155. Hunter CA, Ellis-Neyes LA, Slifer T, et al. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *J Immunol*. 1997;158(7):3311-3316.
156. Pino-Martínez AM, Miranda CG, Batalla EI, González-Cappa SM, Alba Soto CD. IL-10 participates in the expansion and functional activation of CD8+ T cells during acute infection with *Trypanosoma cruzi*. *J Leukoc Biol*. 2019;105(1):163-175.
157. Roffé E, Rothfuchs AG, Santiago HC, et al. IL-10 limits parasite burden and protects against fatal myocarditis in a mouse model of *Trypanosoma cruzi* infection. *J Immunol*. 2012;188(2):649-660.
158. Medina TS, Oliveira GG, Silva MC, et al. Ebi3 prevents *Trypanosoma cruzi*-induced myocarditis by dampening IFN- γ -driven inflammation. *Front Immunol*. 2017;8:1213.
159. Minoprio P, El Cheikh MC, Murphy E, et al. Xid-associated resistance to experimental Chagas' disease is IFN-gamma dependent. *J Immunol*. 1993;151(8):4200-4208.
160. Girard MC, Acevedo GR, Ossowski MS, et al. Altered frequency of CD24high CD38high transitional B cells in patients with cardiac involvement of chronic Chagas disease. *bioRxiv*. 2019;684589.
161. Guthmiller JJ, Graham AC, Zander RA, Pope RL, Butler NS. Cutting edge: IL-10 is essential for the generation of germinal center B cell responses and anti-plasmodium humoral immunity. *J Immunol*. 2017;198(2):617-622.
162. Ferrão PM, d'Avila-Levy CM, Araujo-Jorge TC, et al. Cruzipain activates latent TGF- β from host cells during *T. cruzi* invasion. *PLOS ONE*. 2015;10(5):e0124832.
163. Martin DL, Postan M, Lucas P, Gress R, Tarleton RL. TGF- β regulates pathology but not tissue CD8+ T cell dysfunction during experimental *Trypanosoma cruzi* infection. *Eur J Immunol*. 2007;37(10):2764-2771.
164. Esper L, Roman-Campos D, Lara A, et al. Role of SOCS2 in modulating heart damage and function in a murine model of acute Chagas disease. *Am J Pathol*. 2012;181(1):130-140.
165. Araujo Furlan CL, Tosello Boari J, Rodriguez C, et al. Limited Foxp3+ regulatory T cells response during acute *Trypanosoma cruzi* infection is required to allow the emergence of robust parasite-specific CD8+ T cell immunity. *Front Immunol*. 2018;9:2555.
166. Fresno M, Gironès N. Regulatory lymphoid and myeloid cells determine the cardiac immunopathogenesis of *Trypanosoma cruzi* infection. *Front Microbiol*. 2018;9:351.
167. Carbajosa S, Rodríguez-Angulo HO, Gea S, et al. L-arginine supplementation reduces mortality and improves disease outcome in mice infected with *Trypanosoma cruzi*. *PLoS Negl Trop Dis*. 2018;12(1):e0006179.

168. Parrado R, Ramirez JC, de la Barra A, et al. Usefulness of serial blood sampling and PCR replicates for treatment monitoring of patients with chronic Chagas disease. *Antimicrob Agents Chemother*. 2019;63(2):e01191-18.
169. Bonney KM, Luthringer DJ, Kim SA, Garg NJ, Engman DM. Pathology and pathogenesis of chagas heart disease. *Annu Rev Pathol*. 2019;14(1):421-447.
170. Santi-Rocca J, Fernandez-Cortes F, Chillón-Marinas C, et al. A multi-parametric analysis of *Trypanosoma cruzi* infection: common pathophysiological patterns beyond extreme heterogeneity of host responses. *Sci Rep*. 2017;7(1):1-12.
171. Roffé E, Marino APMP, Weaver J, et al. *Trypanosoma cruzi* causes paralyzing systemic necrotizing vasculitis driven by pathogen-specific type I immunity in mice. *Infect Immun*. 2016;84(4):1123-1136.
172. Cummings KL, Tarleton RL. Rapid quantitation of *Trypanosoma cruzi* in host tissue by real-time PCR. *Mol Biochem Parasitol*. 2003;129(1):53-59.
173. Pereira IR, Vilar-Pereira G, Marques V, et al. A human type 5 adenovirus-based *Trypanosoma cruzi* therapeutic vaccine re-programs immune response and reverses chronic cardiomyopathy. *PLoS Pathog*. 2015;11(1):e1004594.
174. Pack AD, Collins MH, Rosenberg CS, Tarleton RL. Highly competent, non-exhausted CD8⁺ T cells continue to tightly control pathogen load throughout chronic *Trypanosoma cruzi* infection. *PLoS Pathog*. 2018;14(11):e1007410.
175. Ward AI, Lewis MD, Khan AA, et al. In vivo analysis of *Trypanosoma cruzi* persistence foci at single-cell resolution. *mBio*. 2020;11(4):e01242-20.
176. Fiuza JA, Fujiwara RT, Gomes JAS, et al. Profile of central and effector memory T cells in the progression of chronic human Chagas disease. *PLoS Negl Trop Dis*. 2009;3(9):e512.
177. Bustamante JM, Bixby LM, Tarleton RL. Drug-induced cure drives conversion to a stable and protective CD8⁺ T central memory response in chronic Chagas disease. *Nat Med*. 2008;14(5):542-550.
178. Weaver JD, Hoffman VJ, Roffe E, Murphy PM. Low-level parasite Persistence drives vasculitis and myositis in skeletal muscle of mice chronically infected with *Trypanosoma cruzi*. *Infect Immun*. 2019;87(6):e00081-19.
179. Horn D. Antigenic variation in African trypanosomes. *Mol Biochem Parasitol*. 2014;195(2):123-129.
180. Nakayasu ES, Sobreira TJ, Torres R, et al. Improved proteomic approach for the discovery of potential vaccine targets in *Trypanosoma cruzi*. *J Proteome Res*. 2012;11(1):237-246.
181. Llewellyn MS, Messenger LA, Luquetti AO, et al. Deep sequencing of the *Trypanosoma cruzi* GP63 surface proteases reveals diversity and diversifying selection among chronic and congenital Chagas disease patients. *PLoS Negl Trop Dis*. 2015;9(4):e0003458.
182. Kahn SJ, Wleklinski M. The surface glycoproteins of *Trypanosoma cruzi* encode a superfamily of variant T cell epitopes. *J Immunol*. 1997;159(9):4444.
183. Buscaglia CA, Campo VA, Di Noia JM, et al. The surface coat of the mammal-dwelling infective trypomastigote stage of *Trypanosoma cruzi* is formed by highly diverse immunogenic mucins. *J Biol Chem*. 2004;279(16):15860-15869.
184. Bartholomeu DC, Cerqueira GC, Leao ACA, et al. Genomic organization and expression profile of the mucin-associated surface protein (masp) family of the human pathogen *Trypanosoma cruzi*. *Nucleic Acids Res*. 2009;37(10):3407-3417.
185. Barrett MP, Kyle DE, Sibley LD, Radke JB, Tarleton RL. Protozoan persister-like cells and drug treatment failure. *Nat Rev Microbiol*. 2019;17(10):607-620.
186. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. *Nat Rev Microbiol*. 2017;15(8):453-464.
187. Dumoulin PC, Burleigh BA. Stress-induced proliferation and cell cycle plasticity of intracellular *Trypanosoma cruzi* Amastigotes. *mBio*. 2018;9(4):e00673-18.
188. Resende BC, Oliveira ACS, Guañabens ACP, et al. The influence of recombinational processes to induce dormancy in *Trypanosoma cruzi*. *Front Cell Infect Microbiol*. 2020;10:5.
189. Nylén S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends Immunol*. 2007;28(9):378-384.
190. Ferreira RR, da Silva AR, Vilar-Pereira G, et al. TGF- β inhibitor therapy decreases fibrosis and stimulates cardiac improvement in a pre-clinical study of chronic Chagas' heart disease. *PLoS Negl Trop Dis*. 2019;13(7):e0007602.
191. Nogueira LG, Santos RHB, Fiorelli AI, et al. Myocardial gene expression of T-bet, GATA-3, Ror- γ , FoxP3, and hallmark cytokines in chronic Chagas disease cardiomyopathy: an essentially unopposed TH1-type response. *Mediators Inflamm*. 2014;2014:91491426.
192. Salvador F, Sulleiro E, Sánchez-Montalvá A, Martínez-Gallo M, Carrillo E, Molina I. Impact of helminth infection on the clinical and microbiological presentation of chagas diseases in chronically infected patients. *PLoS Negl Trop Dis*. 2016;10(4):e0004663.
193. Albareda MC, Olivera GC, Laucella SA, et al. Chronic human infection with *Trypanosoma cruzi* drives CD4⁺ T cells to immune senescence. *J Immunol*. 2009;183(6):4103-4108.
194. Argüello RJ, Albareda MC, Alvarez MG, et al. Inhibitory receptors are expressed by *Trypanosoma cruzi* -specific effector T cells and in hearts of subjects with chronic Chagas disease. *PLoS One*. 2012;7(5):e35966.
195. Pérez-Antón E, Egui A, Thomas MC, Simón M, Segovia M, López MC. Immunological exhaustion and functional profile of CD8⁺ T lymphocytes as cellular biomarkers of therapeutic efficacy in chronic Chagas disease patients. *Acta Trop*. 2020;202:105242.
196. Tosello Boari J, Araujo Furlan CL, Fiocca Vernengo F, et al. IL-17RA-signaling modulates CD8⁺ T cell survival and exhaustion during *Trypanosoma cruzi* infection. *Front Immunol*. 2018;9:2347.
197. Mateus J, Guerrero P, Lasso P, et al. An animal model of acute and chronic Chagas disease with the reticulotropic Y strain of *Trypanosoma cruzi* that depicts the multifunctionality and dysfunctionality of T cells. *Front Immunol*. 2019;10:918.
198. Quebrada Palacio LP, Fernández ER, Hernández-Vásquez Y, Petray PB, Postan M. Circulating T follicular helper cell abnormalities associated to different clinical forms of chronic Chagas disease. *Front Cell Infect Microbiol*. 2020;10:126.
199. Fernández ER, Olivera GC, Palacio LPQ, et al. Altered distribution of peripheral blood memory B cells in humans chronically infected with *Trypanosoma cruzi*. *PLoS One*. 2014;9(8):e104951.
200. Calderón J, Maganto-García E, Punzón C, Carrión J, Terhorst C, Fresno M. The receptor Slamf1 on the surface of myeloid lineage cells controls susceptibility to infection by *Trypanosoma cruzi*. *PLoS Pathog*. 2012;8(7):e1002799.
201. de Meis J, Morrot A, Farias-de-Oliveira DA, Villa-Verde DMS, Savino W. Differential regional immune response in Chagas disease. *PLoS Negl Trop Dis*. 2009;3(7):e417.
202. McCall L-I, Tripathi A, Vargas F, Knight R, Dorrestein PC, Siqueira-Neto JL. Experimental Chagas disease-induced perturbations of the fecal microbiome and metabolome. *PLoS Negl Trop Dis*. 2018;12(3):e0006344.
203. Silverio JC, Pereira IR, Cipitelli MDC, et al. CD8⁺ T-cells expressing interferon gamma or perforin play antagonistic roles in heart injury in experimental *Trypanosoma cruzi*-elicited cardiomyopathy. *PLoS Pathog*. 2012;8(4):e1002645.
204. Ferreira CP, Cariste LM, Santos Virgilio FD, et al. LFA-1 mediates cytotoxicity and tissue migration of specific CD8⁺ T cells after heterologous prime-boost vaccination against *Trypanosoma cruzi* infection. *Front Immunol*. 2017;8:1291.

205. Pontes Ferreira C, Cariste LM, Ferri Moraschi B, et al. CXCR3 chemokine receptor guides *Trypanosoma cruzi*-specific T-cells triggered by DNA/adenovirus ASP2 vaccine to heart tissue after challenge. *PLoS Negl Trop Dis*. 2019;13(7):e0007597.
206. Leavey JK, Tarleton RL. Cutting edge: dysfunctional CD8+ T cells reside in nonlymphoid tissues during chronic *Trypanosoma cruzi* infection. *J Immunol*. 2003;170(5):2264-2268.
207. Gabanyi I, Muller Paul A, Feighery L, Oliveira Thiago Y, Costa-Pinto Frederico A, Mucida D. Neuro-immune interactions drive tissue programming in intestinal macrophages. *Cell*. 2016;164(3):378-391.
208. La Flamme AC, Kahn SJ, Rudensky AY, Van Voorhis WC. *Trypanosoma cruzi*-infected macrophages are defective in major histocompatibility complex class II antigen presentation. *Eur J Immunol*. 1997;27(12):3085-3094.
209. Alba Soto CD, Mirkin GA, Solana ME, González Cappa SM. *Trypanosoma cruzi* infection modulates in vivo expression of major histocompatibility complex class II molecules on antigen-presenting cells and T-cell stimulatory activity of dendritic cells in a strain-dependent manner. *Infect Immun*. 2003;71(3):1194-1199.
210. Camargo R, Faria LO, Kloss A, et al. *Trypanosoma cruzi* infection down-modulates the immunoproteasome biosynthesis and the MHC class I cell surface expression in HeLa cells. *PLoS One*. 2014;9(4):e95977.
211. Lundberg IE, Grundtman C. Developments in the scientific and clinical understanding of inflammatory myopathies. *Arthritis Res Ther*. 2008;10(5):220.

How to cite this article: Pérez-Mazliah D, Ward AI, Lewis MD. Host-parasite dynamics in Chagas disease from systemic to hyper-local scales. *Parasite Immunol*. 2020;00:e12786. <https://doi.org/10.1111/pim.12786>